

Amendments to the Specification:

Please replace the title as follows:

~~Methods and means for increasing the tolerance of plants to stress conditions.~~

METHODS AND MEANS FOR INCREASING THE TOLERANCE OF PLANTS TO STRESS CONDITIONS

Please insert the following after the title and before "Field of the Invention":

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a U.S. national phase application of International PCT Application No. PCT/EP04/003995, filed April 9, 2004, which claims the benefit under 35 U.S.C. 119(a)-(d) to European Application No. 03076044.1, filed April 9, 2003, and claims the benefit under 35 U.S.C. 119(e) to U.S. Provisional Application No. 60/496,688, filed August 21, 2003, the disclosures of each of which are herein incorporated by reference in their entireties.

BACKGROUND OF THE INVENTION:

Before paragraph [2], please replace the following heading as follows:

~~Background of the Invention~~

Description of the Related Art

Please replace paragraph [2] with the following:

[[.]] Frequently, abiotic stress will lead either directly or indirectly to damage of the DNA of the cells of the plants exposed to the adverse conditions. Genomic damage, if left unrepaired, can lead to cell death. Tolerance to stress conditions exhibited by plants is the result of the ability of the plant cells exposed to the adverse conditions to reduce and/or repair the damage, and to survive.

Please replace paragraph [9] with the following:

PARG encoding genes have been identified in a number of animals such as *Rattus norvegicus* (Accession numbers: NM_031339, NW_043030, AB019366[[.]]), *Mus musculus* (Accession numbers: NT_039598, NM_003631, AF079557), *Homo sapiens* (Accession numbers:

NT_017696; NM_003631, AF005043), *Bos taurus* (Accession numbers: NM_174138, U78975)
Drosophila melanogaster (Accession number: AF079556).

Before paragraph [11], please replace the following heading as follows:

~~Summary of the Invention~~

SUMMARY AND OBJECTS OF THE INVENTION

Before paragraph [18], please replace the following heading as follows:

~~Brief description of the figures~~

BRIEF DESCRIPTION OF THE FIGURES

Before paragraph [21], please replace the following heading as follows:

~~Detailed description of preferred embodiments~~

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Please replace paragraph [40] with the following:

As further described in WO 99/53050, the sense and antisense ParG inhibitory RNA regions, capable of forming a double stranded RNA region may be present in one RNA molecule, preferably separated by a spacer region. The spacer region may comprise an intron sequence. Such a chimeric gene may be conveniently constructed by operably linking a DNA fragment comprising at least 20 nucleotides from the isolated or identified endogenous ParG gene, the expression of which is targeted to be reduced, in an inverted repeat, to a plant expressible promoter and 3'-end 3' end formation region involved in transcription termination and polyadenylation. To achieve the construction of such a chimeric gene, use can be made of the vectors described in WO 02/059294.

Please replace paragraph [66] with the following:

~~SEQ ID N°1:~~ SEQ ID NO 1: amino acid sequence of the ParG protein from *Arabidopsis thaliana*.

Please replace paragraph [67] with the following:

~~SEQ ID N°2:~~ SEQ ID NO 2: amino acid sequence of part of the ParG protein from *Solanum tuberosum*.

Please replace paragraph [68] with the following:

~~SEQ ID N°3:~~ SEQ ID NO 3: nucleotide sequence encoding the ParG protein from *Arabidopsis thaliana*.

Please replace paragraph [69] with the following:

~~SEQ ID N°4:~~ SEQ ID NO 4: nucleotide sequence encoding the part of the ParG protein from *Solanum tuberosum*.

Please replace paragraph [70] with the following:

~~SEQ ID N°5:~~ SEQ ID NO 5: nucleotide sequence of an oligonucleotide primer suitable for PCR amplification of part of a ParG protein encoding DNA fragment.

Please replace paragraph [71] with the following:

~~SEQ ID N°6:~~ SEQ ID NO 6: nucleotide sequence of an oligonucleotide primer suitable for PCR amplification of part of a ParG protein encoding DNA fragment.

Please replace paragraph [72] with the following:

~~SEQ ID N°7:~~ SEQ ID NO 7: nucleotide sequence of an oligonucleotide primer suitable for PCR amplification of part of a ParG protein encoding DNA fragment.

Please replace paragraph [73] with the following:

~~SEQ ID N°8:~~ SEQ ID NO 8: nucleotide sequence of an oligonucleotide primer suitable for PCR amplification of part of a ParG protein encoding DNA fragment.

Please replace paragraph [74] with the following:

~~SEQ ID N°9:~~ SEQ ID NO 9: nucleotide sequence of the T-DNA vector containing the ParG expression reducing chimeric gene based on the *Arabidopsis* ParG gene sequence.

Please replace paragraph [75] with the following:

~~SEQ ID N°10:~~ SEQ ID NO 10: amino acid sequence of conserved sequence 1 of PARG proteins.

Please replace paragraph [76] with the following:

~~SEQ ID N°11:~~ SEQ ID NO 11: amino acid sequence of conserved sequence 2 of PARG proteins.

Please replace paragraph [77] with the following:

~~SEQ ID N°12:~~ SEQ ID NO 12: amino acid sequence of conserved sequence 3 of PARG proteins.

Please replace paragraph [78] with the following:

~~SEQ ID N°13:~~ SEQ ID NO 13: amino acid sequence of conserved sequence 4 of PARG proteins.

Please replace paragraph [79] with the following:

~~SEQ ID N°14:~~ SEQ ID NO 14: amino acid sequence of conserved sequence 5 of PARG proteins.

Please replace paragraph [80] with the following:

~~SEQ ID N°15:~~ SEQ ID NO 15: nucleotide sequence of the ParG protein from *Oryza sativa*.

Please replace paragraph [81] with the following:

~~SEQ ID N°16:~~ SEQ ID NO 16: amino acid sequence of the ParG protein from *Oryza sativa*.

Please replace paragraph [82] with the following:

~~SEQ ID N°17:~~ SEQ ID NO 17: nucleotide sequence of an oligonucleotide primer PG1 suitable for PCR amplification of part of a ParG protein encoding DNA fragment.

Please replace paragraph [83] with the following:

~~SEQ ID N°18:~~ SEQ ID NO 18: nucleotide sequence of an oligonucleotide primer PG2 suitable for PCR amplification of part of a ParG protein encoding DNA fragment.

Please replace paragraph [84] with the following:

~~SEQ ID N°19:~~ SEQ ID NO 19: nucleotide sequence of an oligonucleotide primer PG3 suitable for PCR amplification of part of a ParG protein encoding DNA fragment.

Please replace paragraph [85] with the following:

~~SEQ ID N°20:~~ SEQ ID NO 20: nucleotide sequence of an oligonucleotide primer PG4 suitable for PCR amplification of part of a ParG protein encoding DNA fragment.

Please replace paragraph [86] with the following:

~~SEQ ID N°21:~~ SEQ ID NO 21: nucleotide sequence of an oligonucleotide primer PG5 suitable for PCR amplification of part of a ParG protein encoding DNA fragment.

Please replace paragraph [87] with the following:

~~SEQ ID N°22:~~ SEQ ID NO 22 :nucleotide sequence of an oligonucleotide primer PG6 suitable for PCR amplification of part of a ParG protein encoding DNA fragment.

Please replace paragraph [88] with the following:

~~SEQ ID N°23:~~ SEQ ID NO 23: nucleotide sequence encoding a ParG protein from *Zea mays*.

Please replace paragraph [89] with the following:

~~SEQ ID N°24:~~ SEQ ID NO 24: nucleotide sequence of a T-DNA vector comprising a chimeric gene capable of reducing PARG expression.

Please replace paragraph [90] with the following:

~~SEQ ID N°25:~~ SEQ ID NO 25: nucleotide sequence of a T-DNA vector comprising a chimeric gene capable of reducing PARG expression.

Please replace paragraph [98] with the following:

To reduce the expression of the PARG gene e.g. in *Arabidopsis* and related plants, a chimeric gene was constructed which is capable expressing a dsRNA comprising both a sense and antisense region which can form a double stranded RNA. Such dsRNA is very effective in reducing the expression of the genes with which is shares sequence homology, by post-transcriptional silencing. The chimeric gene comprises the following DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment comprising 163 bp from the ParG gene from *Arabidopsis thaliana* in direct orientation (Genbank Accession number AF394690 from nucleotide position 973 to 1135);

- A DNA fragment encoding intron 2 from the *pdk* gene from *Flaveria*;
- The DNA fragment comprising 163 bp from the *ParG* gene from *Arabidopsis thaliana* in inverted orientation (Genbank Accession number AF394690 from nucleotide position 973 to 1135);
- A fragment of the 3' untranslated end from the octopine synthetase gene from *Agrobacterium tumefaciens*.

Please replace paragraph [100] with the following:

To reduce the expression of the *PARG* gene e.g. in potatoes and related plants, a chimeric gene is constructed which is capable expressing a dsRNA comprising both a sense and antisense region of a cDNA sequence from potato, that is capable of encoding a protein having high sequence identity with the N-terminal part of the *Arabidopsis* *PARG* protein. The chimeric gene comprises the following DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment comprising a sequence of at least 100 bp from *ParG* homologue from *Solanum tuberosum* in direct orientation (Genbank Accession number BE340510);
- A DNA fragment encoding intron 2 from the *pdk* gene from *Flaveria*;
- The DNA fragment comprising the sequence of at least 100 bp from *ParG* homologue from *Solanum tuberosum* in inverted orientation (Genbank Accession number BE340510);
- A fragment of the 3' untranslated end from the octopine synthetase gene from *Agrobacterium tumefaciens*.

Please replace paragraph [115] with the following:

Media and reaction buffers

Sowing medium (medium 201):

Half concentrated Murashige and Skoog salts

2% sucrose

pH 5.8

0.6% agar (Difco Bacto Agar)

250mg/l triacillin

Callus inducing medium A2S3:

MS medium, 0.5g/l Mes (pH 5.8), 3% sucrose, 40mg/l adenine-SO₄, 0.5% agarose,
1mg/l 2,4-D, 0.25mg/l NAA, 1mg/l BAP, 250mg/l triacillin

Incubation medium:

25mM K-phosphate buffer pH5.8
2% sucrose
1 drop Tween20 for 25ml medium

Reaction buffer:

50mM K-phosphate buffer pH7.4
1mM sodium,3'-{1-[phenylamino-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro) =
XTT (bts, Germany, cat n° No. 2525)
1 drop Tween20 for 25ml buffer

Please replace paragraph [120] with the following:

Media and reaction buffers

Plant medium:

Half concentrated Murashige and Skoog salts
B5 vitamins
1.5% sucrose
pH 5.8
0.7% Difco agar

Incubation medium:

10mM K-phosphate buffer pH5.8
2% sucrose
1 drop Tween20 for 25ml medium

Reaction buffer:

50mM K-phosphate buffer pH7.4
1mM sodium,3'-{1-[phenylamino-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro) = XTT (bts, Germany, cat n° No. 2525)
1 drop Tween20 for 25ml buffer

Please replace paragraph [128] with the following:

Transgenic seeds were germinated on a medium containing MS salts/2; B5 vitamins; 1,5% sucrose; ~~pH5.8~~ pH=5.8 and 0.7% Difco agar. Germinated seeds were subject to low light (photosynthetic photon flux of about 30 $\mu\text{mol m}^{-1} \text{s}^{-1}$ for 14 to 18 days, after which the light intensity was increased about 6-fold (photosynthetic photon flux of about 190 $\mu\text{mol m}^{-1} \text{s}^{-1}$). After 1 day, the NAD and NADH contents were determined using the enzymatic cycling method (Karp et al. (1983) Anal. Biochem. 128, pp 175-180). A portion of the seedlings were cultivated further under high light conditions for about 3 to about days, after which the damage was scored. Damage was visible as darkening of the young leaves and shoot tip, bleaching of older leaves and growth retardation. The results are summarized in ~~Table 1~~ Table 2 for Arabidopsis and in ~~Table 2~~ Table 3 for tobacco.

Please replace paragraph [129] with the following:

~~Table 1.~~ Table 2. Analysis of Arabidopsis (Columbia). $\pm R$ indicates that some dark pigmentation was observed. ND: not determined

	High light tolerance	NAD+NADH content in 1 gram of tissue ($10^{-3}\mu\text{M}$)	% TTC-reducing capacity vs control
Non-transgenic control	S	17.3	100
Transgenic line 9	R	28.2	ND
Transgenic line 10	R	31.7	ND
Transgenic line 11	$\pm R$	26.5	ND
Transgenic line 12	S	19.4	ND
Transgenic line 26	R	33.2	55
Transgenic line 27	S	21.3	100

Transgenic line 28	±R	26.5	75
Transgenic line 29	S	17.7	102
Transgenic line 30	R	28.3	66

Please replace paragraph [130] with the following:

~~Table 2.~~ Table 3. Analysis of *Nicotiana tabacum* c.v. Petit Havana SR1. ±R indicates that some dark pigmentation was observed. R/S indicates tha the resistance phenotype was not very clear.

	High light tolerance	% TTC-reducing capacity vs control
Non-transgenic control	S	100
Transgenic line 1	R/S	88
Transgenic line 2	±R	79
Transgenic line 3	R	53